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Synthesis of some novel 3- (4-ethoxyphenyl) -5- (4-substituted) -4,5-dihydro-1H-pyrazole derivatives as potent antioxidant agents

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ABSTRACT

Pyrazolines are the most useful heterocyclic moiety in Pharmaceutical and Chemical fields and as the most potential molecules for the design of new chemical entities. Nitrogen-containing heterocyclic compounds, pyrazolines and their derivatives showed a variety of pharmacological activities, including antioxidant properties. In the present study, eleven novel ethoxylated pyrazoline derivatives were synthesized by condensing chalcones with electron releasing ethoxy group at one end and different electron-donating, electron-withdrawing groups in another end with hydrazine hydrate and alcohol. The compounds synthesized were structural elucidated by their spectroscopic studies. All the compounds synthesized were evaluated for their *in vitro* antioxidant potential by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide free radical scavenging assay methods. Some of these molecules possess moderate to good antioxidant activity when compared to standard ascorbic acid. The compound with methoxy group (EH2) exhibits potent antioxidant activity with IC_{50} value of 9.02 and 9.44μg/ml in DPPH and hydrogen peroxide assay methods respectively and the compound with hydroxy group (EH9) also showed potent antioxidant activity with IC_{50} value of 12.41 and 14.56μg/ml in DPPH and hydrogen peroxide free radical scavenging assay methods respectively when compared to standard. The compounds containing electron-donating substituents were found to be good antioxidants when compared to standard ascorbic acid.

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INTRODUCTION

Pyrazolines are well known five membered heterocyclic compounds with nitrogen and prepared from α, β-unsaturated ketones with nucleophiles like NH_2NH_2. H_2O with alcohol (Sakthinathan *et al.*, 2012; Sharath *et al.*, 2013). Pyrazoline with its derivatives are known to possess different biological and pharmacological activities like anti-inflammatory (Rathish *et al.*, 2009; Prabha *et al.*, 2019), antimicrobial (D'andrea *et al.*, 2005; Prabha *et al.*, 2019), antidepressant (Palaska *et al.*, 1996; Palaska, 2001), antitubercular (Kucukguzel and Rollas, 2002; Shaharyar *et al.*, 2006), antiviral (Kreutzberger and Kolter, 1986), antiamoebic (Abid and Azam, 2005; Abid and Azam,

2006), anticonvulsant (Ozdemir *et al.*, 2007), analgesic (Amir *et al.*, 2008), anticancer (Taj *et al.*, 2011) and antioxidant potential (Martins *et al.*, 2009; Kumar *et al.*, 2013).

Generation of free radicals from the reactions of the imbalance between oxidative stress and antioxidant homeostatic phenomenon, which can leads to many pathological conditions in the body (Tiwari, 2001). An elevated level of oxidative stress is one of the reasons for many diseases. Many biological damage due to the reactions of free radicals like H_2O_2, hydroxyl radicals (OH), oxygen anions (O_2) free radicals, which leads to the process of chain reactions. Much reactive free radical plays a major role in the pathological condition of many diseases like heart disease, cancer, diabetes, strokes, Parkinson's diseases and allergies (Süzen, 2007).

The electron-donating CH_3 cluster substituted in the second position of 2-pyrazoline showed greater antioxidant property, whereas the electron-withdrawing Group (EWG) and high electron-donating groups were not increase free radicals inhibition (Kumar *et al.*, 2013). Indole based pyrazole with an electron-donating group (EDG) has been proved increased antioxidant activity then EWG (Sharath *et al.*, 2013). The presence of 2-quinolone with EWG in a pyrazoline ring showed increased antioxidant activity (Kumar *et al.*, 2016). Nitrogen-containing heterocyclic compounds with the electron-withdrawing nature of halogen groups showed greater antioxidant property (Hossain *et al.*, 2009).

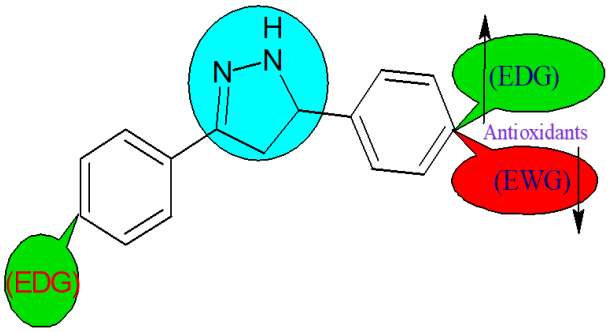


Figure 1: Graphical abstract of the study

On the basis of these considerations, present research work was done to synthesize 1H-pyrazole derivatives with various EDG and EWG and evaluate them for antioxidant potential by DPPH & H_2O_2 assay methods. Graphical abstract of the present study was given in Figure 1.

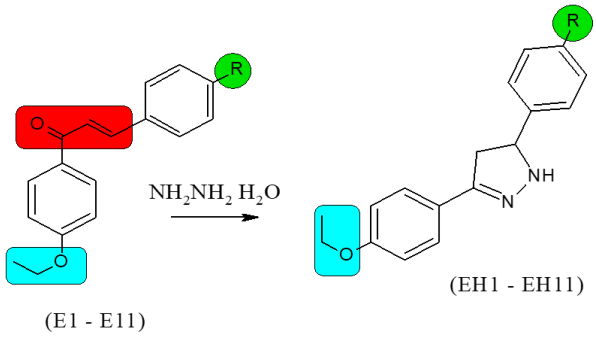
MATERIALS AND METHODS

Chemicals and reagents

Analytical grade of reagent & chemicals were used in this study. All para-substituted aldehydes, hydrogen hydrate and other chemicals were procured from Sigma-Aldrich private limited.

Synthesis and characterization of ethoxylated pyrazolines

Eleven compounds of ethoxylated chalcones were prepared and evaluated their biological activities and reported (Lakshminarayanan *et al.*, 2019). Continuation of the research, all the titled compounds (EH1 – EH11) were obtained by condensation of appropriate chalcones (E1 – E11) with 1.35 ml of hydrazine hydrate in the presence of 60 ml methanol and 15 ml acetic acid. The above mixture refluxed gently in a water bath for 6 – 8 hours. Then cool and acidified with conc. HCl. The product obtained was filtered and washed with water and recrystallized by ethanol. The open capillary method was used to determine the melting point and they were uncorrected. The purity was checked with TLC (hexane: ethyl acetate, 3:7) and melting point test and the route for the synthesis of titled molecules were given in Scheme 1. All prepared molecules were characterized by their spectral studies.



R	R
EH1: H	EH7: 4-F
EH2: 4-OCH3	EH8: 4-Br
EH3: 4-CH3	EH9: 4-OH
EH4: 4-N(CH3)2	EH10: 4-CH2CH3
EH5: 4-NO2	EH11: 4-CF3
EH6: 4-Cl	

Scheme 1: Synthetic route for 1H-pyrazole derivatives

3-(4-ethoxyphenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazole (EH1)

Brown: 54.3 %; m.p: 180-182°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45-1.42 (t, 3H, *J*=12.0 Hz, CH_3-CH_2-O-), 3.93-3.91 (d, 2H, *J*=8.0 Hz, pyrazole H-4), 3.81-3.79 (t, 1H, *J*=8.0 Hz, pyrazole H-5), 4.09-4.07 (q, 2H, *J*=8.0 Hz, CH_3-CH_2-O-), 6.94-6.91 (d, 1H, *J*=12.0 Hz, H_8 & H_{10}), 7.27-7.25 (t, 1H, *J*=8.0

Hz, H_{15}), 7.32 (t, 1H, *J*=12.0 Hz, H_{14} & H_{16}), 7.47 (d, 1H, *J*=12.0 Hz, H_{13} & H_{17}), 7.92 (d, 1H, *J*=12.0 Hz, H_7 & H_{11}), 9.96 (s, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 14.69 (O-CH_2CH_3), 42.46 (C_4), 59.75 (C_5), 63.72 (O-CH_2CH_3), 114.56 (C_8 & C_{10}), 125.50 (C_{15}),127.12 (C_{13}& C_{17}), 127.51 (C_6), 128.13 (C_7& C_{11}), 128.79 (C_{14}& C_{16}). ESI-MS (*m/z*): Calculated- 266.33, Observed- 266.32.

3-(4-ethoxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole (EH2)

Pale brown; Yield: 59.2 %; m.p: 219-221°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45-1.42 (t, 3H, *J*=12.0 Hz, CH_3-CH_2-O-), 3.86 (s, 3H, *J*=10.0 Hz, OCH_3), 3.78 (d, 2H, *J*=8.0 Hz, pyrazole H-4), 3.72 (t, 1H, *J*=8.0 Hz, pyrazole H-5), 4.08 (q, 2H, *J*=8.0 Hz, CH_3-CH_2-O-), 6.94-6.92 (d, 1H, *J*=8.0 Hz, H_8 & H_{10}), 7.28 (d, 1H, *J*=12.0 Hz, H_{14} & H_{16}), 7.41 (d, 1H, *J*=12.0 Hz, H_{13} & H_{17}), 7.94 (d, 1H, *J*=12.0 Hz, H_7 & H_{11}), 9.92 (s, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 15.16 (O-CH_2CH_3), 30.11 (C_4), 64.24 (O-CH_2CH_3), 114.85 (C_8 & C_{10}), 127.32 (C_{13}& C_{17}), 128.58 (C_6), 129.31 (C_7& C_{11}), 114.58 (C_{14}& C_{16}), 130.44 (C_{12}). ESI-MS (*m/z*): Calculated- 296.36, Observed- 296.35.

3-(4-ethoxyphenyl)-5-(4-methylphenyl)-4,5-dihydro-1*H*-pyrazole (EH3)

Pale brown; Yield: 32.1 %; m.p: 194-197°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ:1.45-1.42 (t, 3H, *J*=12.0 Hz, CH_3-CH_2-O-),2.41-2.40 (s, 3H, *J*=4.0 Hz, CH_3), 3.35 (d, 2H, *J*=8.0 Hz, pyrazole H-4),3.75 (t, 1H, *J*=8.0 Hz, pyrazole H-5),4.09-4.07 (q, 2H, *J*=8.0 Hz, CH_3-CH_2-O-),6.91-6.94 (d, 1H, *J*=12.0 Hz, H_8 & H_{10}),7.12 (d, 1H, *J*=12.0 Hz, H_{14} & H_{16}),7.27 (d, 1H, *J*=12.0 Hz, H_{13} & H_{17}),7.66-7.63 (d, 1H, *J*=12.0 Hz, H_7 & H_{11}), 9.94 (s, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 15.09 (O-CH_2CH_3), 21.42 (CH_3), 42.86 (C_4), 60.04 (C_5), 64.06 (O-CH_2CH_3), 114.54 (C_8 & C_{10}), 125.93 (C_{13}& C_{17}), 127.35 (C_6), 128.53 (C_7& C_{11}), 129.02 (C_{14}& C_{16}), 141.33(C_{12}), 161.12 (C_9). ESI-MS (*m/z*): Calculated- 280.36, Observed- 280.35.

4-[3-(4-ethoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl]-N,N-dimethylaniline (EH4)

Brown; Yield: 38.0 %; m.p: 233-235°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.46-1.43 (t, 3H, *J*=12.0 Hz, CH_3-CH_2-O-), 2.42 (s, 3H, *J*=4.0 Hz, CH_3), 3.92 (d, 2H, *J*=8.0 Hz, pyrazole H-4), 3.97 (t, 1H, *J*=8.0 Hz, pyrazole H-5), 4.09-4.07 (q, 2H, *J*=8.0 Hz, CH_3-CH_2-O-), 6.72 (d, 1H, *J*=12.0 Hz, H_{14} & H_{16}), 6.99-6.96 (d, 1H, *J*=12.0 Hz, H_8 & H_{10}), 7.09 (d, 1H, *J*=12.0 Hz, H_{13} & H_{17}), 7.86-7.83 (d, 1H, *J*=12.0 Hz, H_7 & H_{11}), 9.96 (s, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 15.28 (O-CH_2CH_3), 41.51 (CH3), 42.84 (C_4), 52.04 (C_5), 64.16 (O-CH_2CH_3), 111.47 (C_{14}& C_{16}), 114.44 (C_8 & C_{10}), 129.93 (C_{13}& C_{17}), 128.13 (C_6), 128.93 (C_7& C_{11}), 131.32(C_{12}), 162.12 (C_9). ESI-MS (*m/z*): Calculated- 309.40, Observed- 309.39.

3-(4-ethoxyphenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole (EH5)

Yellow: 41.7 %; m.p: 181-183°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.47-1.44 (t, 3H, *J*=12.0 Hz, CH_3-CH_2-O-), 2.53 (d, 2H, *J*=8.0 Hz, pyrazole H-4), 3.15 (t, 1H, *J*=8.0 Hz, pyrazole H-5),4.12 (q, 2H, *J*=8.0 Hz, CH_3-CH_2-O-),6.97 (d, 1H, *J*=12.0 Hz, H_8 & H_{10}), 7.27 (d, 1H, *J*=12.0 Hz, H_{13} & H_{17}), 7.76 (d, 1H, *J*=12.0 Hz, H_7 & H_{11}), 8.23 (s, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 14.27 (O-CH_2CH_3), 63.36 (O-CH_2CH_3), 114.27 (C_8 & C_{10}), 122.33 (C_{13}& C_{17}), 124.13 (C_{14}& C_{16}), 127.55 (C_6), 128.16 (C_7& C_{11}), 146.68 (C_{15}), 148.38 (C_{12}), 153.44 (C_6), 160.32 (C_9). ESI-MS (*m/z*): Calculated- 311.33, Observed- 311.32.

5-(4-chlorophenyl)-3-(4-ethoxyphenyl)-4,5-dihydro-1*H*-pyrazole (EH6)

Brown; Yield: 42.6 %; m.p: 208-210°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.47-1.44 (t, 3H, *J*=12.0 Hz, CH_3-CH_2-O-), 4.07-4.05 (q, 2H, *J*=8.0 Hz, CH_3-CH_2-O-),4.09 (d, 2H, *J*=8.0 Hz, pyrazole H-4), 4.11 (t, 1H, *J*=8.0 Hz, pyrazole H-5), 6.91-6.94 (d, 1H, *J*=12.0 Hz, H_8 & H_{10}), 7.16-7.18 (d, 1H, *J*=8.0 Hz, H_{14} & H_{16}), 7.27-7.29 (d, 1H, *J*=8.0 Hz, H_{13} & H_{17}), 7.68-7.65 (d, 1H, *J*=12.0 Hz, H_7 & H_{11}), 9.97 (s, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 14.69 (O-CH_2CH_3), 42.33 (C_4), 59.21 (C_5), 63.63 (O-CH_2CH_3), 114.61 (C_8 & C_{10}), 127.08 (C_{13}& C_{17}), 128.16 (C_6), 128.97 (C_7& C_{11}), 133.27 (C_{15}), 140.40 (C_{12}), 153.69 (C_3), 160.77 (C_9). ESI-MS (*m/z*): Calculated- 300.78, Observed- 300.77.

3-(4-ethoxyphenyl)-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazole (EH7)

Brown; Yield: 49.4 %; m.p: 201-203°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.43-1.40 (t, 3H, *J*=12.0 Hz, CH_3-CH_2-O-), 3.9 (d, 2H, *J*=8.0 Hz, pyrazole H-4), 3.94 (t, 1H, *J*=8.0 Hz, pyrazole H-5), 4.01-3.99 (q, 2H, *J*=8.0 Hz, CH_3-CH_2-O-),6.95 (d, 1H, *J*=12.0 Hz, H_8 & H_{10}), 7.15 (d, 1H, *J*=8.0 Hz, H_{13} & H_{17}), 7.47 (d, 1H, *J*=8.0 Hz, H_{14} & H_{16}), 7.70 (d, 1H, *J*=12.0 Hz, H_7 & H_{11}), 9.93 (s, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 15.06 (O-CH_2CH_3), 42.95 (C_4), 61.40 (C_5), 64.70 (O-CH_2CH_3), 115.25 (C_8 & C_{10}), 115.80 (C_{14}& C_{16}), 129.05 (C_6), 130.36 (C_7& C_{11}), 130.47 (C_{13}& C_{17}), 131.15 (C_{12}), 163.37 (C_9). ESI-MS (*m/z*): Calculated- 284.32, Observed- 284.31.

5-(4-bromophenyl)-3-(4-ethoxyphenyl)-4,5-dihydro-1*H*-pyrazole (EH8)

Pale yellow; Yield: 41.1 %; m.p: 163-165°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45-1.43 (t, 3H, *J*=8.0 Hz, CH_3-CH_2-O-), 3.69-3.66 (d, 2H, *J*=12.0 Hz, pyrazole H-4), 3.73-3.71 (t, 1H, *J*=8.0 Hz, pyrazole H-5),

4.05-4.03 (q, 2H, *J*=8.0 Hz, CH_3-CH_2-O-), 6.93-6.90 (d, 1H, *J*=12.0 Hz, H_8 & H_{10}), 7.10-7.08 (d, 1H, *J*=8.0 Hz, H_{13} & H_{17}), 7.40-7.42 (d, 1H, *J*=8.0 Hz, H_{14} & H_{16}), 7.67-7.64 (d, 1H, *J*=12.0 Hz, H_7 & H_{11}), 9.94 (s, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 15.11 (O-CH_2CH_3), 42.76 (C_4), 55.55 (C_5), 64.30 (O-CH_2CH_3), 114.38 (C_8 & C_{10}), 121.92 (C_{15}), 127.85 (C_{13}& C_{17}), 128.75 (C_6), 129.85 (C_7& C_{11}), 131.16 (C_{14}& C_{16}), 141.05 (C_{12}), 155.16 (C_3), 161.40 (C_9). ESI-MS (*m/z*): Calculated- 345.23, Observed- 345.22.

4-[3-(4-ethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]phenol (EH9)

Brown; Yield: 52.6 %; m.p: 182-183°C ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45-1.43 (t, 3H, *J*=8.0 Hz, CH_3-CH_2-O-), 3.72-3.70 (d, 2H, *J*=8.0 Hz, pyrazole H-4), 3.77-3.74 (t, 1H, *J*=12.0 Hz, pyrazole H-5), 4.09-4.07 (q, 2H, *J*=8.0 Hz, CH_3-CH_2-O-), 6.93-6.91 (d, 1H, *J*=8.0 Hz, H_{14} & H_{16}), 7.26 (d, 1H, *J*=12.0 Hz, H_{13} & H_{17}), 7.78 (d, 1H, *J*=8.0 Hz, H_8 & H_{10}), 7.94-7.92 (d, 1H, *J*=12.0 Hz, H_7 & H_{11}), 8.03(s, 1H, OH), 9.96 (s, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ:15.07 (O-CH_2CH_3),58.94 (C_5), 64.16 (O-CH_2CH_3), 114.51 (C_8 & C_{10}), 114.64 (C_{14}& C_{16}), 128.87 (C_{13}& C_{17}), 130.79 (C_6), 130.94 (C_7& C_{11}), 131.03 (C_{12}). ESI-MS (*m/z*): Calculated- 282.33, Observed- 282.32.

3-(4-ethoxyphenyl)-5-(4-ethylphenyl)-4,5-dihydro-1*H*-pyrazole (EH10)

Pale yellow; Yield: 61.8 %; m.p: 165-167°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.26-1.24 (t, 3H, *J*= 8.0 Hz, CH_3-CH_2), 1.44-1.42 (t, 3H, *J*=8.0 Hz, CH_3-CH_2-O-), 2.73-2.71 (q, 3H, *J*= 8.0 Hz, CH_3-CH_2), 3.61-3.59 (d, 2H, *J*=8.0 Hz, pyrazole H-4), 3.72-3.69 (t, 1H, *J*=12.0 Hz, pyrazole H-5), 4.05-4.03 (q, 2H, *J*=8.0 Hz, CH_3-CH_2-O-), 7.05-7.03 (d, 1H, *J*=8.0 Hz, H_{14} & H_{16}), 7.23 (d, 1H, *J*=12.0 Hz, H_{13} & H_{17}), 7.08 (d, 1H, *J*=8.0 Hz, H_8 & H_{10}), 7.93-7.91 (d, 1H, *J*=12.0 Hz, H_7 & H_{11}), 9.93 (s, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 14.91 (O-CH_2CH_3), 15.22 (-CH_2CH_3), 28.13 (-CH_2CH_3), 41.71 (C_4), 55.76 (C_5), 64.25 (O-CH_2CH_3), 114.71 (C_8 & C_{10}), 125.32 (C_{13}& C_{17}), 128.21 (C_6), 128.62 (C_7& C_{11}), 129.65 (C_{14}& C_{16}), 140.43(C_{12}), 152.12 (C_3), 161.55 (C_9). ESI-MS (*m/z*): Calculated- 294.39, Observed- 294.38.

3-(4-ethoxyphenyl)-5-[4-(trifluoromethyl)phenyl]-4,5-dihydro-1*H*-pyrazole (EH11)

Pale brown; Yield: 60.9 %; m.p: 189-191°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.44-1.42 (t, 3H, *J*=8.0 Hz, CH_3-CH_2-O-), 3.68-3.65 (d, 2H, *J*=12.0 Hz, pyrazole H-4), 3.53-3.51 (t, 1H, *J*=8.0 Hz, pyrazole H-5), 4.06-4.04 (q, 2H, *J*=8.0 Hz, CH_3-CH_2-O-), 6.99-6.96 (d, 1H, *J*=12.0 Hz, H_8 & H_{10}), 7.18-7.16 (d, 1H, *J*=8.0 Hz, H_{13} & H_{17}), 7.41-7.43 (d, 1H, *J*=8.0 Hz, H_{14} & H_{16}), 7.89-7.86 (d, 1H, *J*=12.0 Hz, H_7 & H_{11}), 9.98 (s, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 15.76 (O-CH_2CH_3), 42.66 (C_4), 52.35 (C_5), 64.33 (O-CH_2CH_3), 114.90 (C_8 & C_{10}), 124.57 (C_{14}& C_{16}), 128.05 (C_6), 128.42 (C_7& C_{11}), 129.46 (C_{13}& C_{17}), 145.16 (C_{12}). ESI-MS (*m/z*): Calculated- 334.33, Observed- 334.32.

In vitro antioxidant activity

DPPH free radical scavenge activity

All titled molecules were selected for their *in vitro* antioxidant potential by scavenging of DPPH free radical (Kaushik *et al.*, 2016). Standard ascorbic acid and synthesized compounds were prepared in different concentrations from 10 to 50 μg/ml from the stock solution (100μg/ml) with distilled carbinol. In an individual test tube, one ml of each compound solution was taken; 4 ml of 0.004% methanol solution of DPPH was added and shaken vigorously. Keep the mixtures in a dark room for 30 minutes at room temperature and measure the absorbance of all the solution at 517 nm using a UV-VISIBLE spectrophotometer (Shimadzu UV-1800). Percentage radical scavenging activity was calculated with the formula [(Ab_0 – Ab_1)/Ab_0] x 100, where Ab_0 is the absorbance of blank, Ab_1 is the absorbance of synthesized compounds/standard. The calibration curve graph was plotted between percent inhibition and concentrations of the test/standard to get the amount of antioxidants need to decrease 50% from the starting concentration of DPPH free radicals. Half maximal inhibition values were calculated from the calibration curve. (Malladi *et al.*, 2014; Kaushik *et al.*, 2015).

Hydrogen peroxide free radical scavenging activity

Hydrogen peroxide free radical scavenging activities of titled compounds were done by the described method with a small modification (Babu *et al.*, 2001; Jayaprakasha *et al.*, 2004). Solutions of 20 mM hydrogen peroxide were prepared in phosphate buffer saline (PBS) and adjust the pH to 7.4. Standard ascorbic acid and synthesized compounds were prepared in different concentrations from 10 - 50 μg/ml with distilled carbinol. Each compound (1 ml) was taken in test tubes and adds 2 ml of H_2O_2 solution to all test tubes. Absorbance was measured at 230 nm after 10 minutes against a blank. The % scavenging activity and IC_{50} values measured and intended by using the formula mentioned in the DPPH assay method.

RESULTS AND DISCUSSION

All the newly synthesized compounds were confirmed by spectroscopic studies.

Table 1: Antioxidant activities of synthesized compounds (EH1 – EH11) against DPPH radical scavenging method

Compounds	% DPPH radical scavenging activity					IC_{50} (μg/ml)
	10 μg/ml	20 μg/ml	30 μg/ml	40 μg/ml	50 μg/ml	
EH1	19.56	19.99	21.47	22.90	23.56	31.84
EH2	50.03	56.72	58.34	61.09	63.56	9.02
EH3	41.21	44.51	48.54	51.35	52.09	25.75
EH4	45.62	48.76	51.82	54.45	55.68	29.02
EH5	40.31	42.68	44.28	45.26	46.74	130.73
EH6	51.25	54.33	58.52	63.00	65.77	36.33
EH7	29.98	32.32	33.65	35.23	36.12	46.56
EH8	29.08	32.17	34.69	36.11	38.49	95.29
EH9	47.17	53.83	55.52	58.12	60.77	12.41
EH10	21.34	22.35	31.12	32.45	34.01	25.67
EH11	19.25	21.6	22.44	24.54	25.97	115.38
Ascorbic acid	46.34	58.90	63.22	67.91	72.76	8.81

Table 2: Antioxidant activities of synthesized compounds (EH1 –EH11) against H_2O_2 radical scavenging method

Compounds	% H_2O_2 radical scavenging activity					IC_{50} (μg/ml)
	10 μg/ml	20 μg/ml	30 μg/ml	40 μg/ml	50 μg/ml	
EH1	40.45	43.09	45.21	46.82	48.00	33.52
EH2	48.61	55.27	59.15	60.21	62.36	9.44
EH3	20.01	22.38	24.51	26.19	27.18	29.53
EH4	37.23	39.02	42.54	44.56	46.32	30.51
EH5	40.01	44.07	46.29	49.52	52.04	117.98
EH6	49.98	51.23	53.51	55.28	57.20	44.70
EH7	18.12	19.90	21.14	22.21	23.25	57.51
EH8	10.04	12.33	14.32	16.48	18.76	100.74
EH9	41.15	50.56	55.34	58.76	60.46	14.56
EH10	40.90	47.17	52.29	55.02	57.04	23.69
EH11	21.91	22.08	23.51	24.19	26.98	102.67
Ascorbic acid	42.45	48.35	51.16	52.89	54.29	7.65

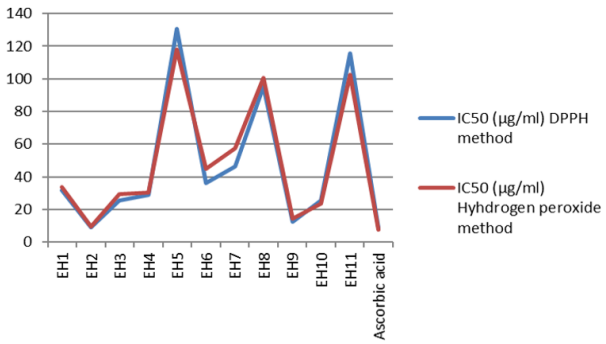


Figure 2: Comparitive IC_{50} values of the synthesized compounds in both assay methods

In vitro antioxidant potency

Newly synthesized compounds (EH1 – EH11) are subjected to evaluate for antioxidant activities by DPPH and H_2O_2 free radicals scavenging methods. Absorbance of DPPH was decreased at 517 nm in all the compounds and standard was observed. The DPPH free radical scavenges activity and 50 % inhibitory concentrations (IC_{50}) for each compound were calculated and depicted in Table 1.

Hydrogen peroxide free radicals are toxic because it may give hydroxyl free radical to the cells even though they were not very reactive. So removal of H_2O_2 radicals is need for antioxidant activities in the

cell. The ability of scavenging H_2O_2 free radicals and 50 % inhibitory concentrations (IC_{50}) for each compound were calculated and depicted in Table 2.

All the compounds showed moderate to good antioxidant potency compared with ascorbic acid. From the results, the compounds EH2 & EH9 with an IC_{50} value of 9.02 and 12.41 in DPPH method and 9.44 & 14.56 in H_2O_2 assay method respectively. These compounds showed very good antioxidant potency among the series when compared to standard ascorbic acid (IC_{50} value of 8.81 in DPPH and 7.65 in H_2O_2 method). It also found from Figure 2 that the synthesized compounds (EH1 – EH11) showed their IC50 values in the DPPH method proportionate to the IC50 values in the hydrogen peroxide assay method.

All the synthesized compounds contains electron-donating –O-CH_2-CH_3 group at one end and different electron-donating and electron-withdrawing groups at another end. Antioxidant potentials of all compounds were influenced by the substituent on an aromatic ring in each compound. The better antioxidant activities of EH2 and EH9 may be owing to the presence of electron giving nature of -OCH_3 and -OH group. On the other hand, the introduction of electron retreating substituents like -Cl, -F, -Br, -CF_3, -NO_2 in compounds EH6, EH7, EH8, EH11 and EH5 has led to the lower the activity when compared with standard. Antioxidant activity of all the compounds may be owed to the presence of electron giving –O-CH_2-CH_3 on one of an aromatic circle in all compounds.

CONCLUSIONS

In conclusion, a new class of nitrogen-containing heterocyclic compounds with the ethoxy group were prepared and screened for their antioxidant potency. Among the compounds, EH2 and EH9 showed better antioxidant activity by both assay methods. Compounds with methoxy and hydroxy groups possess good antioxidant activities when compared to standard.

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